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Role of lipid bodies and lipid-body proteins in seeds and other tissues

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Summary

Lipid bodies are present in most plant cells and not just in storage tissues, such as seeds, as is often assumed. There is a growing number of proteins that are associated with lipid bodies in different plant tissues. In seeds, the most common class of lipid-body protein is the oleosins, which are found on the surfaces of lipid bodies in all desiccation tolerant plants but are absent from lipid bodies in the seeds of desiccation-sensitive plants, e.g. cocoa or neem. The absence of oleosins in these seeds is not problematic during dehydration but leads to a fatal coalescence of lipid bodies after germination. A class of anther-specific proteins has a precursor form that contains an oleosin-like hydrophobic domain and is initially localised on lipid bodies in the tapetum. However, the oleosin-like domain is cleaved off after release into the anther locule and the mature proteins, which are the major protein components of the pollen coat, contain no oleosin-like sequences. Therefore these proteins, which have been named «pollenins», use their oleosin-like domain as a novel form of cleavable targeting sequence. A third type of lipid-body protein is caleosin, which also has endoplasmic reticulum associated isoforms and may be involved in lipid-body biogenesis and membrane trafficking. Analysis of the expression patterns of oleosin and caleosin genes/proteins revealed an unexpected localisation in young root tips, as confirmed by expressing promoter-GUS constructs in transgenic plants. Several other classes of storage product-related genes hitherto believed to seed-specific are also transiently expressed in root tips following seed germination. We discuss the implications of these findings for root development and the use of «seed-specific» gene promoters in general.

Key words: lipid body – oleosin – caleosin – pollenin – seed – root tip

Abbreviations: DAI days after imbibition. – TAG triacylglycerol

Introduction

Lipid bodies in plants are generally thought of in terms of the triacylglycerol (TAG) rich storage organelles found in the

cytoplasm of many oleogenic seeds and fruits. However, there seems little doubt that lipid bodies of various types are widespread, and probably ubiquitous, components of most plant cells. Cytoplasmic lipid bodies can be detected in almost any cell type, even in non-storage tissues of plants – as long as it is at the right stage of development and one

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looks hard enough. Examples include tubers, many types of leaf, roots, root nodules infected with fungi or bacteria, anthers, pollen grains, and bulbs (Murphy 2001). In addition to cytoplasmic lipid bodies, many plant cells also contain lipid bodies in their plastids, including chloroplasts, elaioplasts and chromoplasts (Hernández-Pinzón et al. 1999). Lipid bodies are also formed during senescence, following the scavenging of lipophilic components in plastids (Thompson et al. 1998, Smith et al. 2000).

Seeds are the most common and widespread sites for the accumulation of storage lipid bodies in plants. It is likely that all seeds accumulate at least some cytoplasmic lipid bodies and even non-oilseed species, such as peas, contain about 2% of their seed weight as neutral lipid (Coxon and Davies 1982). True oilseeds accumulate lipid bodies as one of their major storage reserves in amounts ranging from about 20% seed weight in soybean to 42% in rapeseed and as high as 76% in some of the larger seeded nuts (Murphy 1996). Lipid bodies are found in several different types of seed tissue, e.g. in albuminous dicotyledons like castor bean and coriander seeds they accumulate mainly in the endosperm (Moloney 1999); in exalbuminous dicotyledons like rapeseed and sunflower they are found mainly in the embryo cotyledons and axis (Huang 1992, Murphy 1990, 1993); while in monocotyledons such as cereals they may be located in the embryo scutellum (Qu et al. 1986) and/or in the aleurone layer (Aalen et al. 1994). Seed lipid bodies are organelles for the long-term storage of neutral lipids that are eventually mobilised after germination. In seeds that undergo desiccation (see below) a single cell may contain dozens of lipid bodies of only about 0.5–1.0 µm diameter and even non-desiccating seeds contain numerous slightly larger lipid bodies ranging from 2–10 µm in diameter.

It has been known for over a decade that the lipid bodies of many seeds are surrounded by a specific class of proteins termed oleosins. Nevertheless, there is increasing evidence that seeds from tropical or subtropical plants that do not normally undergo dehydration either have a reduced oleosin content or may lack these proteins altogether (Leprince et al. 1998). This raises the question of the role of oleosins in plants since they are apparently not required for lipid-body biogenesis or mobilisation, which both proceed normally in those seeds that lack oleosins. More recently a novel class of lipid-body proteins, termed caleosins, have been described in a wide range of plants and fungi (Naested et al. 2000). In this article, the composition, biogenesis and function of plant lipid bodies and their associated proteins, i.e. oleosins, pollenins and caleosins, will be examined. Although the main focus is on seed lipid bodies, other tissues will also be discussed for comparison including some surprising observations concerning the expression in developing root tips of genes hitherto regarded as seed-specific.

Oleosins: lipid-body proteins in seeds and root tips

Oleosins are relatively low molecular weight proteins of about 16–24 kDa that accumulate on the surfaces of lipid bodies in desiccation tolerant seeds (Huang 1996, Murphy and Vance 1999). Oleosins are characterised by a central hydrophobic domain of about 70 residues that is flanked by more polar N- and C-terminal domains. The widespread occurrence of oleosins and their high abundance in oilseeds (<10–20% total seed protein) has led to suggestions that they may be involved in the formation of lipid bodies from the ER membrane during seed development and/or lipid-body mobilisation following seed germination. Another common suggestion is that oleosins may stabilise lipid bodies during the dehydration stage of seed maturation when all but a few percent of water is lost. Recent evidence cast doubts on all of these proposals and implies that oleosins play no part in lipid-body biogenesis but may function during post-germinative rehydration of seeds (Leprince et al. 1998). This has come from an analysis of the behaviour of lipid bodies during normal or forced dehydration and rehydration in a variety of temperate and tropical/subtropical seeds as summarised in Table 1.

The seeds of many tropical and subtropical plants retain high moisture contents even after release and dispersal. Such seeds are then able to germinate rapidly without undergoing a period of dormancy and rehydration. These non-desiccating, or «recalcitrant», species include important several oilseed crops, such as cocoa, and their lack of tolerance to drying or low temperature has seriously hindered efforts aimed at the long-term storage of such seeds, either for breeding or in germplasm collections (Pritchard et al. 1995). Lipid bodies in these seeds tend to be significantly larger than those of desiccating seeds, e.g. about 7 µm diameter in the cotyledons of mature cocoa seeds, and these lipid bod-

Table 1. Correlation of presence of oleosins with responses of seed lipid bodies (LB) to desiccation and rehydration (Leprince et al., 1998)

Species	Dehydration- seed moisture content ¹	LB ² integrity	Rehydration- seed moisture content	LB integrity	Oleosin ³
<i>C. arabica</i> coffee	11%	+	11–33%	+	83
<i>S. setigera</i> shea	50–2%	+	3–48%	+	73
<i>A. indica</i> neem	73–6%	+	6–57%	-	7
<i>T. cacao</i> cocoa	60–6%	+	6–56%	-	0

¹percentages refer to seed water content; ²lipid-body integrity was assayed by electron microscopy; ³oleosins were assayed by SDS-PAGE and protein sequencing

ies also contain undetectable levels of oleosins (Leprince et al. 1998). Interestingly, the seeds of so-called «intermediate» species, such as neem, coffee and red oak, which have some ability to dehydrate but are still very sensitive to low temperatures, contained intermediate sized lipid bodies (2–5 µm) and quite low levels of putative oleosins per TAG (2–80 %) relative to rapeseed, as shown in Table 1.

In all cases, the lipid bodies of the tropical or subtropical seeds were stable to artificial dehydration, but in recalcitrant seeds like cocoa there was a catastrophic fusion of lipid bodies immediately upon rehydration. In an independent study it was also reported that both water and low-temperature stress led to fusion of lipid bodies in cocoa seeds (Ruhl et al. 1995). These authors noted fusion of lipid bodies during seed dehydration but this is likely to have been a secondary effect of the aqueous fixation buffer used prior to microscopy since when similarly treated seeds were fixed using low-temperature fields-emission scanning EM no such fusion was observed (Leprince et al. 1998). Lipid bodies in non-desiccating seeds probably do not fuse during artificial drying because of the dramatic increase in viscosity as soluble cytoplasmic components, such as sugars and proteins, become more concentrated (Leprince et al. 1998). This is consistent with the observation that, whereas no lipid-body fusion occurred after rapid drying (which generates higher viscosities), slower drying did result in some limited fusion (Leprince et al. 1998). It can be concluded that an important and possibly the principal role for oleosins is indeed to stabilise lipid bodies, but that physiologically this is more relevant during seed imbibition than desiccation.

Pollenins: oleosin-like precursor proteins

These proteins are encoded by anther-specific genes and were originally named oleosins or oleosin-like proteins due to the presence of a 70-residue domain that is homologous with the central hydrophobic lipid-binding domain of seed oleosins (Lee et al. 1994, Robert et al. 1994, Roberts et al. 1993, Ross and Murphy 1996). It was originally believed that these proteins were also present on cytoplasmic lipid bodies in pollen grains (Roberts et al. 1993), although this is no longer thought to be the case (Murphy and Ross 1998, Ross and Murphy 1996). More recent evidence suggests that the oleosin-like region may simply be a novel type of cleavable targeting motif as it is not present in the mature polypeptide (Hernández-Pinzón et al. 1998, Murphy and Ross 1998, Ross and Murphy 1996, Ting et al. 1998). Since the mature forms of these proteins constitute the main protein component of the pollen coat in many species of the Brassicaceae and since they have no resemblance to oleosins, it has been suggested that they be renamed as «pollenins» (Murphy 2001). The pollenins are an interesting class of diverse structural proteins that appear to use tapetal lipid bodies and oleosin-like do-

main as a novel type of non-secreted extracellular targeting mechanism.

Pollenins, or oleosin-like genes, are a large class of anther-specific genes expressed in the tapetal cells of rapeseed and related species (Robert et al. 1994, Murphy and Ross 1998, Ross and Murphy 1996). The encoded proteins contain a 70-residue oleosin-like domain at the N-terminus followed by a C-terminal ranging in length from 7–37 kDa that often contains motifs characteristic of structural proteins (Ross and Murphy 1996, Murphy and Ross 1998). Genes encoding similar proteins have been described in *Arabidopsis* (de Oliveira et al. 1993), which also provides a clue as to the origin of pollenins. One of the *Arabidopsis* pollenin genes, termed atgrp-8 (de Oliveira et al. 1993), is made up of an oleosin-like exon separated by an intron from a second exon encoding a C-terminal domain that is 90 % similar to a separate gene, termed atgrp-4, that is expressed preferentially in roots and stems (de Oliveira et al. 1990). Both atgrp-4 and the C-terminal domain of atgrp-8 are glycine-rich proteins that are predicted to adopt extended conformations, such as anti-parallel β -sheets, that are often found in cell wall proteins (Ross and Murphy 1996). In all the sequenced pollenin genes where introns are present, these are located exactly at the junction of the oleosin-like and C-terminal coding regions. Therefore it is possible that pollenins arose by exon shuffling, whereby genes encoding a variety of structural polypeptides acquired additional exons encoding the hydrophobic central domain of seed oleosins. Although pollenins were initially described only in the Brassicaceae, recent evidence that there are homologous genes in olives (Alche et al., pers. comm.) implies that they may be widespread in higher plants.

Pollenins accumulate initially as full-length proteins (i.e. with the oleosin-like domain still present) in tapetal cells, in association with the cytoplasmic lipid bodies, termed tapetosomes. Isolation of tapetosomes from rapeseed anthers has confirmed that the pollenins remain attached to these organelles as intact full-length proteins, including the oleosin-like domains, throughout tapetal development (Hernández-Pinzón et al. 1998, Ting et al. 1998). However, once the tapetal cells disintegrate and the tapetosomes are released into the anther locule, the pollenins are proteolytically cleaved at the junction of the oleosin-like domain and the C-terminal domain (Hernández-Pinzón et al. 1998, Ting et al. 1998). This coincides with a reorganisation of the tapetosomes, together with the lipid-rich elaioplasts and other tapetal cell contents, to form the material, termed the «pollen coat», which constitutes the outer coating of the pollen grains (Piffanelli and Murphy 1998, Piffanelli et al. 1998, Hernández-Pinzón et al. 1998, Ting et al. 1998).

Analysis of the proteome of the pollen coat of rapeseed showed that all eight of the most abundant polypeptides were mature pollenins, i.e. the C-terminal domains of the original full-length precursor proteins (Murphy and Ross 1998). Only tiny traces of the oleosin-like domains were found in the pollen coat, indicating that these fragments are largely de-

graded after cleavage and play no further part in pollen function. In contrast, the mature, oleosin-free, pollenins have recently been shown to be required for normal rapid initiation of pollination in *Arabidopsis*, since a mutation that removed one of the major pollenins resulted in a severe impairment of pollen hydration and competitive ability after germination (Mayfield and Preuss 2000).

In summary, it appears that pollenins are hybrid proteins made up of a 7–8 kDa lipid-binding domain, similar to the central hydrophobic region of seed oleosins, that has become fused to any one of a wide variety of structural protein domains ranging from 7–37 kDa. The full-length pollenin proteins probably associate with, and possibly stabilise via their oleosin-like domain, the unique cytoplasmic lipid bodies of tapetal cells, i.e. the tapetosomes. It is only when the tapetal cells undergo apoptosis that the tapetosomes are released from the cells and come into contact with proteases that remove the now-redundant oleosin-like domain to leave the mature pollenins to function as the major protein components of the pollen coat. The exact role of pollenins, although evidently important, is still unclear. It seems likely that they are involved in pollen rehydration, possibly by facilitating the creation of water channels through the otherwise relatively impermeable lipidic pollen coat. The reason for the diversity of pollenins may be that there are many alternative combinations of amino acid sequences that can form water channels and that some redundancy is desirable in such an important process as pollen germination. In this case the oleosin-like domains in effect act as a signal sequences that tether pollenins to the lipid bodies to ensure their delivery to the pollen wall at the appropriate stage of development.

Caleosins: lipid-body and ER proteins

Caleosins are a recently discovered class of proteins that were independently named as such by two different groups by virtue of the presence of a single conserved EF-hand calcium-binding domain and an oleosin-like association with lipid bodies (Chen et al. 1999, Naested et al. 2000). These proteins were initially discovered as gene products that were expressed in developing and germinating seeds of rice in response to abscisic acid or to osmotic stress (Frandsen et al. 1996). Caleosin genes are present in a wide range of plants, plus several lipid-accumulating fungi and the single-celled alga, *Chlorella protothecoides* (Naested et al. 2000). Very recent findings have emphasised that although there are many intriguing similarities between caleosins and oleosins, there are also important differences. For example, there are at least seven caleosin genes in *Arabidopsis* but only one of the encoded proteins is tightly bound to lipid bodies during seed development; other isoforms accumulate in vegetative tissues and are probably integral components of the ER membrane as described below (Hernández-Pinzón et al. 2001).

The key structural features of caleosins are an N-terminal region with a single Ca^{2+} -binding EF hand domain, a central hydrophobic domain with a potential lipid-binding region, and a C-terminal region with conserved protein kinase phosphorylation sites. Caleosins contain a central hydrophobic domain of some 30 residues, which is somewhat shorter than the analogous 70-residue domain of oleosins. Caleosins also contain a proline-rich region with the potential to form a «proline knot» motif of the type that appears to be so important in the lipid-body targeting of oleosins (Abell et al. 1997). In addition to the hydrophobic and proline rich domains, caleosins also possess an immediately adjacent potential amphipathic α -helical domain, e.g. residues 129–165 of AtCLO1 in Naested et al. (2000), which may play a role in their binding both to bilayer membranes and to lipid bodies.

Very recently we have discovered that developing seeds contain two molecular weight forms of caleosin that can be distinguished by their subcellular localisation (Murphy et al. 2000, Hernández-Pinzón et al. 2001). Early in seed development, before the onset of storage lipid accumulation, only a 27 kDa ER-localised isoform is present. As lipid bodies are formed a 25 kDa isoform that is exclusively located on the lipid-body surface gradually accumulates. In dry seeds, which contain a much reduced endomembrane system, the 27 kDa ER-isoform is virtually undetectable but it then reappears as the network is re-established after germination. At the same time, the 25 kDa lipid-body isoform disappears in parallel with the mobilisation of the seed storage lipid. Whereas the 25 kDa lipid-body isoform is only found in seeds and root tips, the 27 kDa ER-isoform is also found in roots, stems and young leaves (Murphy et al. 2000, Naested et al. 2000).

These results allow us to tentatively suggest some possible functions of caleosins in seeds and other tissues. Caleosins may be involved in processes such as membrane and lipid-body fusion. Ca^{2+} -mediated fusion has been shown to be a key aspect of the maturation of microlipid bodies released from the ER to produce the large cytoplasmic lipid bodies characteristic of animal lipid-storing cells (Valivullah et al. 1988, Murphy and Vance 1999). Likewise, in seeds and other storage-lipid accumulating plant tissues, nascent lipid-bodies are released as small droplets from the ER and then undergo several rounds of fusion to produce the mature 0.4–2 μm diameter lipid bodies characteristic of such tissues (Huang 1996, Sarmiento et al. 1997). The structural conservation of the Ca^{2+} -binding and phosphorylation domains of caleosins, which are motifs often found in signal-transducing proteins, imply that caleosin function could be modulated by Ca^{2+} -binding and phosphorylation in the cell.

We found that caleosins persisted as integral lipid-body proteins throughout seed desiccation, dormancy and for at least the first six days of post-germinative seedling development. It has been suggested that lipid bodies may dock with glyoxysomes to facilitate the concerted lipolysis and gluconeogenesis that occurs during storage lipid mobilisation (Chapman and Trelease 1991) and caleosins may play a role

in this lipid trafficking process. The microsomal caleosins found in very young embryos and in other plant tissues such as roots and leaves may be involved in other membrane-fission and/or fusion events relating to trafficking between the ER and storage or transport vesicles. Nearly all other characterized EF-hand proteins contain one or more pairs of EF-hands that each cooperatively bind two Ca^{2+} atoms with high affinity, whereas single EF-hand domains only bind a single Ca^{2+} atom with relatively low affinity (Ikura 1996, Kawasaki et al. 1998). It is therefore possible that a caleosin monomer forms dimeric or *cis* associations with another caleosin in the same membrane or lipid body in order to establish the pair of EF-hands required for high affinity Ca^{2+} binding. Alternatively, caleosins on one membrane or lipid body may form *trans* associations with caleosins on a different membrane or lipid body in order to form pairs of EF-hands. Such putative *trans* interactions have analogies with v-SNARE/t-SNARE and tethering factor interactions that are involved in membrane fusion in all eukaryotic cells (Mayer 1999), including those of plants (Sanderfoot and Raikhel 1999).

Root tips and their surprising similarities with seeds

The 25 kDa lipid-body bound isoform of caleosin (Clo-1) is encoded by a gene which we and others initially believed to be seed-specific based on Northern blotting with gene-spe-

cific probes (Chen et al. 1999, Naested et al. 2000). This was further checked by making a construct consisting of the *Arabidopsis* Clo-1 promoter driving the GUS reporter gene and expressing it in transgenic *Arabidopsis* plants. Following histochemical analysis we observed the expected high levels of GUS activity in developing embryo cotyledons but were surprised to see almost equally high GUS staining in one additional tissue, i.e. the root tip of seedlings shortly after germination (Naested et al. 2000). All of the transformants exhibited exactly the same pattern of seed-specific and early root tip-specific GUS activity, which makes it less likely to be a transformation artefact. In the same study we observed that immuno-detectable oleosin was expressed in rapeseed root tip cells, and that caleosin is associated with it on what appeared to be lipid bodies. This raised the question of whether oleosin (and maybe other "seed-specific" genes) are also expressed in root tips.

We therefore re-examined the GUS expression patterns in transgenic *Brassica napus* plants containing a *B. napus* oleosin promoter driving the GUS gene. To our further surprise we found high levels of GUS activity in the root-tip region of seedlings at 2 DAI (days after imbibition) as shown in Fig. 1. Apart from root tips, GUS activity was confined to the expected location, i.e. developing seeds. The same expression patterns were found in several independent transgenic lines, which means that this phenomenon is unlikely to be due to a transformation artefact. However, one characteristic of the GUS enzyme is its great stability and there is a

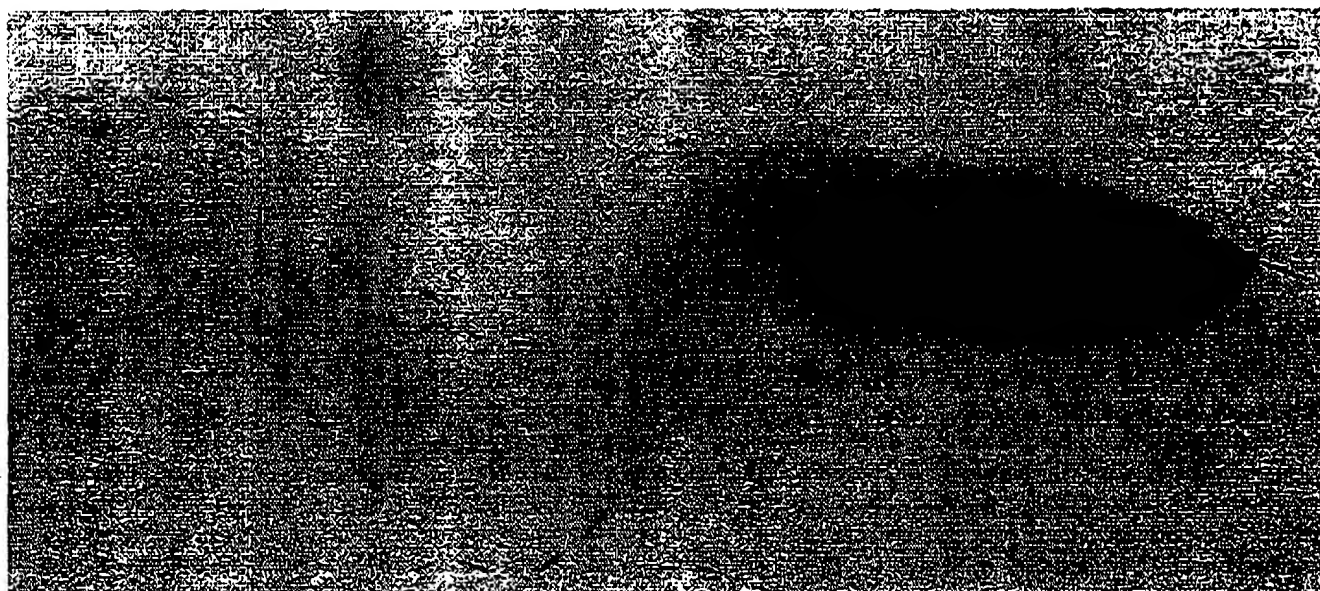


Figure 1. Histochemical staining of GUS enzyme activity in the roots of transgenic *Brassica napus* seedlings containing an oleosin promoter-(OP)-GUS construct, as described in Keddie et al. (1994). The GUS activity is confined to the root tip region and was not present in any other part of the root or in any other tissue of the seedlings in any of the transgenic lines that were analysed. The dashed lines indicate the outline of the non-stained part of the root.

Table 2. Relative abundance of oleosin mRNA in different tissues of developing seeds and germinated seedlings of *B. napus* as determined by Northern hybridisation with a full-length oleosin cDNA probe as described by Sarmiento et al. (1997). Note that expression is confined to developing seeds and 2 DAI root tips.

Tissue	Torpedo	Early Cotyledon	Mid Cotyledon	Late Cotyledon	2 Mature	4 DAI*	6 DAI*	8 DAI*
Cotyledon	-	■	■ ■	■ ■ ■ ■	-	-	-	-
Root tip					■ ■	■ ■ ■	-	-
Stem					-	-	-	-

*DAI, days after imbibition

slight possibility that the GUS activity in root tips resulted from enzyme that was originally synthesised during seed development and persisted after germination. If this did occur, however, the GUS activity should have been seen throughout the seedling, whereas we found it to be confined to a small region of the root tip. Also, we only detected GUS staining at 2 DAI and there was no trace by 4 DAI. Nevertheless, we also checked directly whether oleosin gene expression was detectable in root tips and other tissues by Northern blotting. The results of several blots are summarised in Table 2 and demonstrate conclusively that the oleosin gene is indeed expressed at moderately high levels (comparable to those in mid-cotyledonary embryos) in root tips. It must be emphasised that this expression is transient as both the Northern blotting and the GUS staining patterns showed that the oleosin gene expression was confined to root tips at the first few days after imbibition – no oleosin mRNA or GUS activity was detected either earlier (in dry seeds) or later (after 4 DAI).

These data raise the question «why are these genes encoding lipid-body-related proteins expressed in root tips?» Interestingly, the presence of lipid bodies has been reported previously in root tips or root caps of rice (Sargent and Osborne 1980), pea and maize (Craig and Staehelin 1988) and garden cress (Hensel 1986, Busch et al. 1993). It has also been reported that lipid bodies in root tip cells from garden cress concentrate calcium (Busch et al. 1993), which would be consistent with the presence of caleosin on their surfaces. Therefore root tips may behave in a similar manner to seeds in accumulating, albeit transiently, lipid bodies. These lipid bodies may have a storage function, as in seeds, but may have different functions in root tips. For example, during their initial period of differentiation, root cap cells act as statocytes (gravity sensors). In root cap statocytes of cress there are abundant lipid bodies that appear to determine a polar distribution of ER, i.e. preferentially at the distal cell pole (Hensel 1986). Hence, at least in cress, the lipid bodies may be one component of the positive orthogravitropic growth of roots. This has interesting analogies with the proposed gravity-

sensing role of lipid bodies in the motile algal raphidophyte *Heterosigma akashiwo* (Wada et al. 1987) and the fungus *Phycomyces blakesleeana* (Schimek et al. 1999).

Further evidence for the expression in root tips of genes hitherto regarded as seed-specific has come from several sources. For example the promoter of the *Vicia faba* seed storage protein, legumin B4, drove GUS expression in both developing seeds and young root tips in transgenic tobacco plants (Bäumlein and Wobus, pers. comm.). Another gene from *Vicia faba*, that was named USP (for unknown seed protein), also shows very high expression in developing seeds but also a transient activity in root tips (Bäumlein et al. 1991). The USP promoter has been used to drive a variety of different transgenes, including single-chain antibodies (Fielder et al. 1997) and is regulated by the embryo maturation-related transcription factor, FUS3 (Reidt et al. 2000), which makes it transient expression in root tips even more interesting. There are additional anecdotal accounts of such seed + root tip expression patterns that are known to the authors but which are normally dismissed as artefacts and therefore not reported. In view of the findings presented here, some of these observations may well bear more detailed investigation.

The emerging picture is that root tips in several species transiently express genes, such as oleosins and storage proteins, which are normally associated with the storage product accumulation stage of seed development. This is puzzling since the root tip contains a very active meristematic region that would not normally be associated with the accumulation of storage products, particularly in such a transient fashion. Therefore, while root tips may accumulate storage products like lipid bodies and protein bodies (although the presence of protein bodies has yet to be established), the purpose of these structures may be different from their role in seeds. One particularly intriguing possibility relates to gravity sensing as discussed above. The observation that both storage lipid and storage protein related genes are expressed in root tips is particularly noteworthy in view of the reported combined roles of «sedimenting protein crystals and floating lipid globules» in gravitropism in the fungus, *Phycomyces blakesleeana* (Schimek et al. 1999).

Another important consequence of these findings is that at least some of the supposedly seed-specific gene promoters, including napin, USP and oleosin, that are currently in use in transgenic crops probably also direct expression of transgenes in root-tips with uncertain phenotypic consequences for the development of such plants. A more detailed analysis of such transgene expression patterns should therefore form part of the risk assessment protocol for all transgenic plants expressing genes directed by «seed-specific» promoters.

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